

GnTV was cloned from human gene (cDNA) as follows. The primers used for amplification by PCR were GnTVF: agagtcgacatggctctcttcactccgtgg (SEQ ID NO:9) containing an added *Sal* I site and GnTVR17: tgaggtaccctataggcagtcctttgc (SEQ ID NO:11) containing an added *Kpn* I site.--

Please delete the original sequence listing filed March 5, 2002, page numbered 26 - 29.
Please insert the attached Substitute Sequence Listing, independently numbered pages 1 - 4 directly after the abstract.

IN THE CLAIMS:

Please change the page numbers containing the claims from page numbers 30-31 to 26-27.

REMARKS


Enclosed herewith in full compliance with 37 C.F.R. §§1.821-1.825 is a Substitute Sequence Listing to be inserted into the specification as indicated above. The Substitute Sequence Listing in no way introduces new matter into the specification. Also submitted herewith in full compliance with 37 C.F.R. §§1.821-1.825 is a disk copy of the Substitute Sequence Listing. The disk copy of the Substitute Sequence Listing, file "0760-0303P.ST25.TXT", is identical to the paper copy, except that it lacks formatting.

SEQ ID NOS have been added to the Specification to identify them and place them in the proper format. SEQ ID NOS:12-15 have been added to the Substitute Sequence Listing. SEQ ID NOS:12-13 have been added for the primers found on page 13 of the specification.

SEQ ID NOS:14-15 have been added for the amino acid sequences found on page 6 of the specification. No new matter is introduced by these amendments.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,
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Attachments: Disk Copy of Substitute Sequence Listing
Paper Copy of Substitute Sequence Listing
Copy of Notification
Version with Markings Showing Changes Made

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The paragraph beginning on page 5, line 6, has been amended as follows:

--The desired protein is not restricted at all and any protein may be employed. Preferred examples of the desired protein include glycosyltransferases such as fucosyltransferases 1 to 9, N-acetylglucosaminyltransferases I to IV, sialic acid transferases and galactosyltransferases; sulfotransferases which transfer sulfate group to sugar chains of glycolipids (e.g., heparan sulfate N-sulfotransferase and cerebroside sulfotransferase which synthesizes galactosylceramide sulfate); and the entire type II membrane proteins including scavenger receptor family including LDL oxide scavenger receptors and macrophage receptor with collagenous structure (MACRO), but the desired proteins are not restricted thereto. In the fusion gene containing the gene encoding the protein constituting the virus particle and the gene encoding the desired protein, the two genes may be directly ligated or may be indirectly ligated through an intervening sequence (in this case, the gene located at the downstream region should be in-frame (i.e., the reading frames of the two genes are coincide) with the other gene located at the upstream region). Thus, the term "fusion protein" used in the specification and claims of the present application includes both cases wherein the desired protein is directly ligated to the virus particle-constituting protein and wherein the desired protein is indirectly ligated to the virus particle-constituting protein through an intervening region. The fusion gene containing the two genes may be first formed and the formed fusion gene may be inserted into the vector. Alternatively, one of the two genes may be first inserted into the vector and then the other gene is inserted into the vector to form the fusion gene in the vector. By ligating the two genes through an intervening region encoding the

sequence Leu-Val-Gly-Arg-Pro-Ser (SEQ ID NO:14) recognized by thrombin or the sequence Ile-Glu-Gly-Arg (SEQ ID NO:15) recognized by Factor Xa, the desired protein may easily be separated from the virus particle by treating the fusion protein with thrombin or Factor Xa.--

The paragraph beginning on page 13, line 11, has been amended as follows:

-- α (1,3/1,4) fucosyltransferase gene (GenBank Accession No. X53578, hereinafter referred to as "FUT3") was introduced into baculovirus for producing FUT3 protein as follows. The FUT3 gene was cloned from human gene (cDNA) according to a conventional method. The primers used in PCR for amplifying the gene were FUT3F1: tcg cat atg gat ccc ctg ggt gca gcc aag (SEQ ID NO:12) containing an added *Nde* I site and FUT3R3: atg ctcgag tca ggt gaa cca agc cgc tat (SEQ ID NO:13) containing an added *Xho* I site. The PCR product of FUT3 gene and the constructed pFB6A/CCR3 (see Reference Example 2 below) were treated with restriction enzymes *Nde* I and *Xho* I. These were ligated and introduced into *E. coli* cells (DH5 α competent cells). The resulting cells were plated on an ampicillin-containing LB agar plate and cultured at 37°C for about 16 hours. From this plate, a single *E. coli* colony was selected and the selected *E. coli* cells were cultured in ampicillin-containing culture medium for about 16 hours under shaking. Plasmids were recovered from the grown *E. coli* cells and the inserted FUT3 gene was sequenced. As a result, the determined sequence was identical to the reported sequence of the FUT3 gene (GenBank Accession No. X53578). This plasmid containing the inserted FUT3 gene was named pFB6A/FUT3.--

The paragraph beginning on page 15, line 13, has been amended as follows:

--By the conventional method, mRNAs were extracted from human leukocytes, cDNAs were prepared therefrom, and chemokine receptor CCR3 gene (cDNA) was cloned. In this operation, the gene excluding the termination codon was amplified by PCR. The primers used for the PCR had added restriction enzyme recognition sites. That is, the used primers were CCR3F: tcgcatatgacaacctcactagatacagtt (SEQ ID NO:1) and CCR3R: tgcggaattcaaacacaatagagagttccggctctg (SEQ ID NO:2). The PCR product of the CCR3 gene was treated with restriction enzymes *Nde* I and *Eco* RI. The plasmid (hereinafter referred to as "pFB6A") used in the cloning was the same as pFastBac donor plasmid (commercially available from GibcoBRL) except that the multicloning site was modified to contain *Nde* I and *Eco* RI restriction sites. The plasmid pFB6A was also treated with *Nde* I and *Eco* RI, and the resultant was ligated with the PCR product of the CCR3 gene treated with the restriction enzymes.--

The paragraph beginning on page 16, line 9, has been amended as follows:

--To the CCR3 gene in the constructed plasmid pFB6A/CCR3, $\alpha(1,3/1,4)$ fucosyltransferase gene, which is a glycosyltransferase, was ligated so as to obtain CCR3-FUT3 fusion protein as follows. As the FUT3 gene, the one cloned in Reference Example 1 was used. PCR was performed using a primer FUT3F: tgcggaattcatggatcccctgggtgcagcc (SEQ ID NO:3) containing an added *Eco* RI site and a primer FUT3R: tgtctcgagtcaggtgaaccaagccgctat (SEQ ID NO:4) containing an added *Xho* I site. The PCR product of the FUT3 gene and the constructed pFB6A/CCR3 plasmid were digested with restriction enzymes *Eco* RI and *Xho* I.

The obtained digests were ligated by a conventional method and the resultant was introduced into *E. coli* cells (DH5 α competent cells). The cells were plated on an ampicillin-containing LB agar plate and incubated at 37°C for about 16 hours. A single *E. coli* colony was selected from this plate and the selected *E. coli* was cultured in ampicillin-containing LB medium for about 16 hours under shaking. Plasmids were extracted from the grown *E. coli* and the inserted FUT3 gene was sequenced. As a result, the sequence was identical to the reported FUT3 gene (GenBank Accession No. X53578). The obtained plasmid into which the FUT3 gene was inserted was named pFB6A/CCR3-FUT3.--

The paragraph beginning on page 18, line 4, has been amended as follows:

--By the conventional method, genomic DNA was extracted from baculovirus, and gp64 gene was cloned. In this operation, the gene excluding the termination codon was amplified by PCR. The primers used for the PCR had restriction enzyme recognition sites. That is, the used primers were gp64F: tcgcatatggtaagcgctattgttttatat (SEQ ID NO:5) containing an added *Nde* I site and gp64R: tgcgaattcatattgtctattacggtttct (SEQ ID NO:6) containing an added *Eco* RI site. The PCR product of the gp64 gene was treated with restriction enzymes *Nde* I and *Eco* RI. The plasmid used in the cloning was pFB6A. The plasmid pFB6A was also treated with *Nde* I and *Eco* RI, and the resultant was ligated with the PCR product of the gp64 gene treated with the restriction enzymes.--

The paragraph beginning on page 21, line 9, has been amended as follows:

--For producing a fusion protein containing CCR3, CCR3 gene was cloned in pFastBac donor plasmid 1 (hereinafter referred to as "pFB1", commercially available from GibcoBRL) after changing the sequence of the multicloning site as follows. The CCR3 gene excluding the termination codon was amplified by PCR. The primers used in the PCR contained added restriction sites. That is, the primers used were CCR3FE: tcggaattcatgacaacctcactagataca (SEQ ID NO:7) containing an added *Eco* RI site, and CCR3RS: tgcgtcgaccaaacacaatagagagtcc (SEQ ID NO:8) containing an added *Sal* I site. The PCR product of the CCR3 gene and the pFB1 plasmid were digested with restriction enzymes *Eco* RI and *Sal* I, and the digests were ligated by a conventional method.--

The paragraph beginning on page 22, line 1, has been amended as follows:

--To ligate N-acetylglucosaminyltransferase V gene (GenBank Accession No. NM002410, hereinafter referred to as "GnTV"), which is a glycosyltransferase, to the CCR3 gene in the constructed plasmid pFB1/CCR3, GnTV gene was cloned. The GnTV was cloned from human gene (cDNA) as follows. The primers used for amplification by PCR were GnTVF: agagtcgacatggctctctcactccgtgg (SEQ ID NO:9) containing an added *Sal* I site and GnTVRXho: tgactcgagctataggcagttcttgc (SEQ ID NO:10) containing an added *Xho* I site. The PCR product of the GnTV gene and the plasmid pFB1/CCR3 were digested with restriction enzymes *Sal* I and *Xho* I. The digests were ligated by a conventional method and the resulting plasmid was introduced into *E. coli* cells (DH5 α competent cells). The cells were plated on an ampicillin-containing LB agar plate and incubated at 37°C for about 16 hours. A single *E. coli*

colony was selected from this plate and the selected *E. coli* was cultured in ampicillin-containing LB medium for about 16 hours under shaking. Plasmids were extracted from the grown *E. coli* and the inserted GnTV gene was sequenced. As a result, the sequence was identical to the reported GnTV gene (GenBank Accession No. NM002410). The obtained plasmid into which the GnTV gene was inserted was named pFB1/CCR3-GnTV.--

The paragraph beginning on page 23, line 24, has been amended as follows:

--To ligate gp64 gene and N-acetylglucosaminyltransferase V gene, which is a glycosyltransferase, for obtaining gp64-GnTV fusion protein, GnTV gene was cloned. The GnTV was cloned from human gene (cDNA) as follows. The primers used for amplification by PCR were GnTVF: agagtcgacatggctctcttcactccgtgg (SEQ ID NO:9) containing an added *Sal* I site and GnTVR17: tgaggtaccctataggcagctctttgc (SEQ ID NO:11) containing an added *Kpn* I site.--